Product :
ETHYL VIOLET AZIDE BROTH (EVA BROTH)

Also known as
Litsky Broth; Azide-Ethyl Violet Broth

Specification
Liquid medium for the confirmation of enterococci in water.

Formula * in g/L
Meat peptone..... 10.0000
Casein peptone..... 10.0000
Dextrose..... 5.0000
Sodium chloride..... 5.0000
Monopotasium phosphate..... 2.7000
Dipotassium phosphate..... 2.7000
Sodium azide..... 0.3000
Ethyl violet..... 0.0005

Final pH 7,0 ±0,2 at 25 ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions
Dissolve 35.6 g of powder in 1 L of distilled water, heating slightly if necessary. Distribute in tubes or flasks and sterilize in the autoclave at 121ºC for 15 minutes.

Description
EVA Broth is a highly selective medium for some enterococci, and it has been adopted by many Official Organisations, national and international. The medium's high selectivity is due to the presence of Sodium Azide and Ethyl Violet, as they inhibit other accompanying bacteria, blocking their respiratory chains, at the same time leaving enterococci unaffected. In general, this medium is used as a confirmation medium in the second stage, with an inoculum from a suitable medium such as Rothe Azide Broth (Art. No. 02-027) inoculated in this medium.

Technique
Each of the EVA Broth tubes is inoculated with one or two loops from a presumed positive Rothe Azide Broth, and is incubated for a 24-48 hours period at 36±1°C. The presence of Enterococcus is demostrated by turbidity in the medium.
Occasionally a slight turbidity may appear accompanied by abundant violet sediment at the bottom of the tube.
Commonly, growth in this medium is considered enough to confirm the prensence of enterococci.
However, confirmative identification must be carried out by isolation in solid media and classification in one of the four faecal enterococci species: Enterococcus faecalis, Enterococcus faecium, Enterococcus bovis and Enterococcus equinum.

Quality control
Incubation temperature: 36°C ± 2,0 **Incubation time:** 44 ± 4h

Inoculum: Pure cultures using and inoculating needle

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC® 25923	Inhibited	-
Escherichia coli ATCC® 25922	Inhibited	-
Enterococcus faecalis ATCC® 29212	Good to very good	Violet precipitate
Enterococcus faecalis ATCC® 19433	Good to very good	Violet precipitate

References

· CLESCERI, L., A.E. GREENBERG & E.A. EATON (1998) Standard Methods for the Examination of Water and Wastewater. APHA-AWWA-WEF, 20th ed., Washington.
· DOWNES, F.C. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
· GUINEA, SANCHO & PARÉS (1979) Análisis Microbiológico de Aguas: Aspectos Aplicados. Ed. Omega. Barcelona.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· LITSKY,W., W.L. MALLMAN & C.W. FIFIELD (1953) A New Medium for the Detection of Enterococci in Water . Amer. J. Publ. Hlth 43(7):873.
· ROTHE (1948) Illinois State Health Department.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).